

## **APPENDIX B**

# Taxonomy and Metabolism of *Lactobacillus*

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## Introduction

Today taxonomy is often based on a polyphasic approach (Vandamme *et al.*, 1996a), which involves genotypic and phenotypic methods. Pure historically phenotypic methods have dominated the identification and classification schemes of the lactic acid bacteria and the lactobacilli in particular. Today, 16S rRNA sequencing has become the method of choice, not only because of its high degree of portability, but equally important, because of the availability of a large database of reference sequences. In a polyphasic approach, which involves both genotypic and phenotypic methods, one may encounter differences between, for example, the phylogenetic tree revealed by 16S rRNA sequencing and the phenotypic groups based on fermentation profiles and metabolite production.

It would be wrong, however, to limit all classification schemes to the phylogenetic groupings based on 16S rRNA sequences exclusively. 16S rRNA suffers from considerable disadvantages, as will be shown below. A polyphasic approach that involves a balanced use of multiple genotypic and phenotypic methods will always yield a more balanced and reliable result. As sequencing becomes more and more accessible and cheaper, the role of a single molecule such as 16S rRNA, will tend to fade. The sequencing of a variety of individual (household) genes will diversify and broaden the taxonomic views, with the sequencing of the complete genome of many organisms as a feasible option within a few years.

Today, however, 16S rRNA in combination with DNA-DNA hybridizations is still the ref-

erence method. Therefore, the taxonomic discussion in this chapter will be based on a neighbour joining tree, obtained with the 16S rRNA of the *Lactobacillus* species known at the end of May 2008.

In the section 'Metabolism' below, we describe the remarkable variation of catabolic activities within the genus *Lactobacillus*. This variation, together with the fact that lactobacilli are generally considered safe, has been the basis of their very broad use in food applications (Vankerckhoven *et al.*, 2008; Huys *et al.*, 2006). The active application of living microorganisms in foods requires proper labelling (Temmerman *et al.*, 2004), which makes proper identification, based on stable and reliable classification schemes, extremely important.

## Metabolism

The first essential step in food fermentations is the catabolism of carbohydrates by the lactic acid bacteria. Lactic acid bacteria as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds. The main end product is lactic acid (>50% of sugar carbon). It should be noticed, however, that lactic acid bacteria adapt to various conditions and change their metabolism accordingly. This may lead to significantly different end product patterns (Table 2.1).

The taxonomy of lactic acid bacteria, for many decades heavily relied on the type of sugar fermentation. In order to deal with the large number of species being described, Oda-Jensen (1919, 1942, 1943) proposed a classification

Table 2.1 Historical subdivision of the genus *Lactobacillus* according to the type of fermentation

	I: Homofermentative	II: Heterofermentative
Glucose fermented to lactic acid	≥ 85%	60%
Formation of $\text{CO}_2$ , acetic acid and ethanol	-	+
$\text{CO}_2$ formed from glucose	-	+
Thiamine required for growth	-	+
Fructose diphosphate aldolase	+	-
Orla-Jensen, 1919		
	<i>'Thermobacterium'</i> / <i>'Streptobacterium'</i>	<i>'Betabacterium'</i>
	Obligate homofermentative	Facultative heterofermentative
		Obligate heterofermentative
van den Hamer, 1960		
Fructose-1,6-bisphosphate aldolase	+	+
Glucose-6-phosphate dehydrogenase	-	+
6-Phosphogluconate dehydrogenase	-	+
Rogosa, 1970	IA	IB
Growth at 45°C	+	d
Growth at 15°C	-	d
Ribose fermented	-	+
$\text{CO}_2$ from gluconate	-	+
Rogosa, 1974		
Acidophilic		
Ethanol tolerant		
Most carbohydrates fermented		

of lactic acid bacteria, which was based on morphology, temperature range of growth, nutritional characteristics, carbon sources utilization and agglutination effects. Orla-Jensen differentiated three major groups. The first group contained *Thermobacterium*, *Streptobacterium* and *Streptococcus*, which were all catalase negative and produce mainly lactic acid besides traces of other by-products. The second group contained

*Betabacterium* and *Betacoccus*, which also lack catalase but as a rule formed detectable amounts of gas and other by-products, besides lactic acid. The third group consisting of *Microbacterium* and *Tetracoccus* show a positive catalase reaction.

In 1960, van den Hamer showed that representatives of *Betabacterium* did not possess fructose-1,6-bisphosphate aldolase, in contrast to *Thermobacterium* and

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 Table 2.1 *continued*

	I: Homofermentative	II: Heterofermentative	
		IIA	IIB
Sharpe, 1979			
Aerobic species	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus casei</i>	<i>Lactobacillus brevis</i>
	<i>Lactobacillus delbrueckii</i>	<i>Lactobacillus coryniformis</i>	<i>Lactobacillus buchneri</i>
	<i>Lactobacillus helveticus</i>	<i>Lactobacillus curvatus</i>	<i>Lactobacillus confusus</i>
	<i>Lactobacillus jensenii</i>	<i>Lactobacillus homothochii</i>	<i>Lactobacillus fermentum</i>
	<i>Lactobacillus salivarius</i>	<i>Lactobacillus plantarum</i>	<i>Lactobacillus viridescens</i>
		<i>Lactobacillus yamanshensis</i>	
Anaerobic species	<i>Lactobacillus ruminis</i>		
	<i>Lactobacillus vitulinus</i>		
Kandler and Weiss, 1986			
	Group I	Group II	Group III
Hexose almost exclusively to lactic acid	+	+	-
Hexose fermented to lactic-, acetic acid, ethanol, $\text{CO}_2$	-	-	+
Lactic-, acetic-, formic acid, ethanol under glucose limitation	d	+	+
Pentose phosphoketolase	-	+	+
Gluconate fermented	-	+	+

d, strain dependent; L, *Lactobacillus*.

*Streptobacterium*. These findings supported the discrimination of three physiological groups: (i) the obligately homofermentative lactobacilli, lacking both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (*Thermobacterium*), (ii) the facultatively homofermentative lactobacilli, having both dehydrogenases, but degrading glucose, preferably via the Embden-Meyerhof-Parnas pathway (*Streptobacterium*), and (iii) the obligately heterofermentative lactobacilli, lacking fructose-1,6-bisphosphate-aldolase (*Betabacterium*). Ther-

mobacterium, *Streptobacterium* and *Betabacterium* were considered to be the three subgenera within the genus *Lactobacillus*.

The subdivision of lactobacilli into three major fermentation groups for taxonomic reasons was maintained until the late 1970s (Table 2.1). The development and application of advanced molecular techniques brought new insights in the taxonomy of the genus, which is meanwhile considered the most heterogeneous among the lactic acid bacteria, with currently 113 different species described (Table 2.2). However, for practical rea-

Table 2.2 List of *Lactobacillus* species with some details on their original description and their current taxonomic status. The table also contains some information on their respective phylogenetic position, metabolism and peptidoglycan type

Number	Genus	Species	Subspecies	References	Cult
1	<i>Lactobacillus</i>	<i>acetotolerans</i>		Entani et al. 1988	
2	<i>Lactobacillus</i>	<i>acidifumaris</i>		Vancanneyt et al. 2005b	
3	<i>Lactobacillus</i>	<i>acidipiscis</i>		Tanasupawat et al. 2000	
4	<i>Lactobacillus</i>	<i>acidophilus</i>		(Moro 1900) Hansen and Mocquot 1970; Johnson et al. 1980	
5	<i>Lactobacillus</i>	<i>agilis</i>		Weiss et al. 1981, 1982	
6	<i>Lactobacillus</i>	<i>algidus</i>		Kato et al. 2000	
7	<i>Lactobacillus</i>	<i>alimentarius</i>		(ex Reuter 1970) Reuter 1983a,b	
8	<i>Lactobacillus</i>	<i>amylolyticus</i>		Bohak et al. 1998, 1999	
9	<i>Lactobacillus</i>	<i>amylophilus</i>		Nakamura and Crowell 1979, 1981	
10	<i>Lactobacillus</i>	<i>amylotrophicus</i>		Naser et al. 2006c	
11	<i>Lactobacillus</i>	<i>amylorovorus</i>		Nakamura 1981	
12	<i>Lactobacillus</i>	<i>animalis</i>		Dent and Williams 1982, 1983	
13	<i>Lactobacillus</i>	<i>anti</i>		Roos et al. 2005	
14	<i>Lactobacillus</i>	<i>apodemii</i>		Osawa et al. 2006	
	<i>Lactobacillus</i>	" <i>artznerensis</i> "		Swezey et al. 2000	
15	<i>Lactobacillus</i>	<i>avarius</i>		Fujisawa et al. 1984, 1985	
	<i>Lactobacillus</i>	<i>avarius</i>	<i>braffinosis</i>	Fujisawa et al. 1984, 1986	
	<i>Lactobacillus</i>	<i>avarius</i>	<i>avarius</i>	Fujisawa et al. 1984, 1986	
	<i>Lactobacillus</i>	" <i>backi</i> "		Bohak et al., 2006	No
	<i>Lactobacillus</i>	" <i>bavaricus</i> "		Slater and Stetter 1980	Lac
	<i>Lactobacillus</i>	" <i>bifementans</i> "		(ex Petta and van Beynum 1943) Kandler et al. 1983b,c	Cell
16	<i>Lactobacillus</i>	<i>brevis</i>		(Orla-Jensen 1919) Bergey et al. 1934	
17	<i>Lactobacillus</i>	<i>buchneri</i>		(Henneberg 1903) Bergey et al. 1923	
	<i>Lactobacillus</i>	" <i>bulgaricus</i> "		(Orla-Jensen 1919) Rogosa and Hansen 1971	Lac
18	<i>Lactobacillus</i>	<i>camelliae</i>		Tanasupawat et al. 2007	bul
	" <i>Lactobacillus</i> "	" <i>camis</i> "		Shaw and Harding 1985, 1986	Cam
19	<i>Lactobacillus</i>	<i>casei</i>		(Orla-Jensen 1916) Hansen and Lessel 1971	(Co)
	<i>Lactobacillus</i>	" <i>casei</i> "	" <i>lactosus</i> "	Mills and Lessel 1973	Lac
	<i>Lactobacillus</i>	<i>casei</i>	<i>casei</i>	(Orla-Jensen 1916) Hansen and Lessel 1971	par
	<i>Lactobacillus</i>	" <i>casei</i> "	" <i>pseudoplantarum</i> "	Abo-Elnaga and Kandler 1965b	Se
	<i>Lactobacillus</i>	" <i>casei</i> "	" <i>hammosus</i> "	Hansen 1968	Lac
	<i>Lactobacillus</i>	" <i>casei</i> "	" <i>toletans</i> "	Abo-Elnaga and Kandler 1965b	par
20	<i>Lactobacillus</i>	<i>catenaformis</i>		(Eggerth 1935) Moore and Holdeman 1970	Se
	<i>Lactobacillus</i>	" <i>cellulosus</i> "		Rogosa et al. 1953	Lac
21	<i>Lactobacillus</i>	<i>cellulotolerans</i>		Vela et al. 2008	par
22	<i>Lactobacillus</i>	<i>coleohominis</i>		Nikolaitchouk et al. 2001	Se
23	<i>Lactobacillus</i>	<i>compositi</i>		Erido and Okada 2007a	Lac
24	<i>Lactobacillus</i>	<i>coifferoides</i>		Cart and Davies 1972	par

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
<i>acidophilus</i>	B	35-37	Lys-D-Asp	DL	
<i>buchneri</i>	C	51	NA	DL	
<i>salivarius</i>	B	38-41	Lys-D-Asp	L	
<i>acidophilus</i>	A	34-37	Lys-D-Asp	DL	
<i>salivarius</i>	B	43-44	DAP	L	
<i>salivarius</i>	B	36-37	DAP	L	
<i>plantarum</i>	B	36-37	Lys-D-Asp	L-DL	
<i>acidophilus</i>	A	39	Lys-D-Asp	DL	
<i>acidophilus</i>	A	44-46	Lys-D-Asp	L	
<i>acidophilus</i>	A	43.5	NA	L	
<i>acidophilus</i>	A	40-41	Lys-D-Asp	DL	
<i>salivarius</i>	A	41-44	Lys-D-Asp	L	
<i>reuteri</i>	C	44-45	Lys-D-Asp	DL	
<i>salivarius</i>	B	38.5	L-Lys-D-Asp	L	
<i>Lactobacillus plantarum</i> (Kostinek et al. 2005)	<i>plantarum</i>	B	48	NA	DL
	<i>salivarius</i>	A	39-43	Lys-D-Asp	DL
	<i>salivarius</i>	A	39-43	Lys-D-Asp	DL (D <15%)
	<i>salivarius</i>	A	39-43	Lys-D-Asp	DL
Not yet validated	NA	NA	NA	NA	NA
<i>Lactobacillus sakei</i> (Kagermeier-Callaway and Lauer 1995)	<i>casei</i>	B	41-43	Lys-D-Asp	L
	<i>casei</i>	B	45	Lys-D-Asp	DL
	<i>buchneri</i>	C	44-47	Lys-D-Asp	DL
	<i>buchneri</i>	C	44-46	Lys-D-Asp	DL
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> (Weiss et al. 1983b)	<i>acidophilus</i>	A	49-51	Lys-D-Asp	D
	<i>casei</i>	A	51.9	Lys-D-Asp	L
<i>Cannobacterium maltaromaticus</i> (Collins et al. 1987)					
	<i>casei</i>	B	45-47	Lys-D-Asp	L
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> (Collins et al. 1989b)	<i>casei</i>	B	45-47	Lys-D-Asp	L
See text for further explanation	<i>casei</i>	B	45-47	Lys-D-Asp	L
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> (Collins et al. 1989b)	<i>casei</i>	B	45-47	Lys-D-Asp	L
<i>Lactobacillus rhamnosus</i> (Collins et al. 1989b)	<i>casei</i>	B	45-47	Lys-D-Asp	L
<i>Lactobacillus paracasei</i> subsp. <i>tolerans</i> (Collins et al. 1989b)	<i>casei</i>	B	45-47	Lys-D-Asp	L
	<i>vitulinus- catenaformis</i>	A	31-33	Lys-Ala	D
<i>Lactobacillus fermentum</i> (Dellaglio et al. 2004a)	<i>reuteri</i>	C	53	Orn-D-Asp	L or DL
	<i>salivarius</i>	B	NA	Lys-L-Ser	L
	<i>reuteri</i>	B	NA	mDAP	DL
	<i>perolens</i>	C	46	Lys-D-Asp	DL
	<i>plantarum</i>	B	48	no mDAP	DL

Table 2.2 continued

Number	Genus	Species	Subspecies	References
25	<i>Lactobacillus</i>	<i>concavus</i>		Tong and Dong 2005
	" <i>Lactobacillus</i> "	<i>confusus</i>		(Holzapfel and Kandler 1969) Sharpe et al. 1972
26	<i>Lactobacillus</i>	<i>coryniformis</i>		Abo-Elnaga and Kandler 1965b
		<i>coryniformis</i>	<i>coryniformis</i>	Abo-Elnaga and Kandler 1965b
		<i>coryniformis</i>	<i>torquens</i>	Abo-Elnaga and Kandler 1965b
27	<i>Lactobacillus</i>	<i>crispatus</i>		(Byngoe and Aladama 1953) Moore and Holdeman 1970
28	<i>Lactobacillus</i>	<i>curvatus</i>		(Trolli-Pettersson 1903) Abo-Elnaga and Kandler 1965b emend. Klein et al. 1996
		<i>curvatus</i>	<i>curvatus</i>	Torriani et al. 1996
		<i>curvatus</i>	" <i>melibiosus</i> "	Torriani et al. 1996
	<i>Lactobacillus</i>	" <i>cypriacae</i> "		Lawson et al. 2001a
29	<i>Lactobacillus</i>	<i>delbrueckii</i>		(Leichmann 1896) Beijerinck 1901
		<i>delbrueckii</i>	<i>bulgaricus</i>	(Oria-Jensen 1919) Weiss et al. 1983b, 1984
		<i>delbrueckii</i>	<i>delbrueckii</i>	(Leichmann 1896) Beijerinck 1901
		<i>delbrueckii</i>	<i>indicus</i>	Dellaglio et al. 2005
		<i>delbrueckii</i>	<i>lactis</i>	(Oria-Jensen 1919) Weiss et al. 1983b, 1984
30	<i>Lactobacillus</i>	<i>diolivorans</i>		Kroonen et al. 2002
	<i>Lactobacillus</i>	" <i>disidiosus</i> "		Vaughn, et al. 1949
	" <i>Lactobacillus</i> "	<i>divergens</i>		Holzapfel and Gerber 1983, 1984
	<i>Lactobacillus</i>	" <i>duriensis</i> "		Leisner et al. 2002
31	<i>Lactobacillus</i>	<i>equi</i>		Morotomi et al. 2002
32	<i>Lactobacillus</i>	<i>fercimini</i>		(ex Reuter 1970) Reuter 1983a,b
33	<i>Lactobacillus</i>	<i>farinoris</i>		Endo and Okada 2007b
	<i>Lactobacillus</i>	" <i>termitoshensis</i> "		Simpson et al. 2001, 2002
34	<i>Lactobacillus</i>	<i>fermentum</i>		Beijerinck 1901 emend. Dellaglio et al. 2004a
35	<i>Lactobacillus</i>	<i>fornicatus</i>		Dicks et al. 2000
36	<i>Lactobacillus</i>	<i>fructivorans</i>		Charlton et al. 1934
	" <i>Lactobacillus</i> "	<i>fructosus</i>		Kodama 1956
37	<i>Lactobacillus</i>	<i>frumenti</i>		Möller et al. 2000
38	<i>Lactobacillus</i>	<i>fuchuensis</i>		Sakala et al. 2002
39	<i>Lactobacillus</i>	<i>gallinarum</i>		Fujisawa et al. 1992
40	<i>Lactobacillus</i>	<i>gasseri</i>		Lauer and Kandler 1980
41	<i>Lactobacillus</i>	<i>gastricus</i>		Roos et al. 2005
42	<i>Lactobacillus</i>	<i>ghenensis</i>		Nielsen et al. 2007
43	<i>Lactobacillus</i>	<i>graminis</i>		Beck et al. 1988, 1989
	" <i>Lactobacillus</i> "	" <i>halotolerans</i> "		Kandler et al. 1983a,c
44	<i>Lactobacillus</i>	<i>hammarii</i>		Valcheva et al. 2005
45	<i>Lactobacillus</i>	<i>hamstenii</i>		Mitsuoka and Fujisawa 1987, 1988
46	<i>Lactobacillus</i>	<i>harbinensis</i>		Miyamoto et al. 2005, 2006
47	<i>Lactobacillus</i>	<i>hayakawai</i>		Morita et al. 2007

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Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
<i>Weissella confusa</i> (Collins et al. 1993)	perolens	A	45-47	mDAP	DL (D 5%)
	casel	B	45	Lys-D-Asp	DL
	casel	B	45	Lys-D-Asp	DL (L<15%)
	casel	B	45	Lys-D-Asp	D
	acidophilus	A	35-38	Lys-D-Asp	DL
	casel	B	42-44	Lys-D-Asp	DL
	casel	B	42-44	Lys-D-Asp	DL
	casel	B	42-44	Lys-D-Asp	DL
<i>Lactobacillus sakei</i> subsp. <i>carnosus</i> (Koort et al. 2004)	salivarius	B	40	NA	L
<i>Lactobacillus acidipiscis</i> (Naser et al. 2006b)	acidophilus	A	49-51	Lys-D-Asp	D
	acidophilus	A	49-51	Lys-D-Asp	D
	acidophilus	A	49-51	Lys-D-Asp	D
	acidophilus	A	NA	NA	D
	acidophilus	A	49-51	Lys-D-Asp	D
	buchneri	C	NA	NA	NA
	buchneri	C	NA	NA	NA
<i>Lactobacillus kefir</i> (Marshall et al. 1984).					
<i>Cannobacterium divergens</i> (Collins et al., 1987)					
<i>Lactobacillus vaccinostercus</i> (Delaglio et al. 2005)	reuteri	C	43	NA	DL
	salivarius	A	38-39	NA	DL
	plantarum	A	34-36	Lys-D-Asp	L (L<15%)
	buchneri	B	40-41	no mDAP	DL
<i>Lactobacillus parabuchneri</i> (Vancanneyt et al. 2005a).	buchneri	C	43	NA	DL
	reuteri	C	52-54	Om-D-Asp	DL
	acidophilus	B	37	NA	DL
	buchneri	C	38-41	Lys-D-Asp	DL
<i>Leuconostoc fructosum</i> (Antunes et al. 2002)					
	reuteri	C	43-44	Lys-D-Asp	L
	casel	B	41-42	NA	L (D<40%)
	acidophilus	A	36-37	Lys-D-Asp	DL
	acidophilus	A	33-35	Lys-D-Asp	DL
	reuteri	C	41-42	L-Om-D-Asp	DL
	salivarius	A	38	mDAP	DL
	casel	B	41-43	Lys-D-Asp	DL
<i>Lactobacillus viridescens</i> subsp. <i>halotolerans</i> ; <i>Weissella halotolerans</i> (Collins et al. 1993,1994)					
	buchneri	B	NA	L-Lys-D-Asp	DL
	acidophilus	B	33-35	Lys-D-Asp	DL
	perolens	B	53-54	NA	L
	salivarius	A	34	Lys-Asp	L

Table 2.2 continued

Number	Genus	Species	Subspecies	References
48	<i>Lactobacillus</i>	<i>helveticus</i>		(Orla-Jensen 1919) Bergey et al. 1925
	<i>Lactobacillus</i>	"heteroholchii"		Kitahara et al. 1957a,b; Mōmose et al. 1974
49	<i>Lactobacillus</i>	<i>hilgardii</i>		Douglas and Cruess 1936
50	<i>Lactobacillus</i>	<i>homoholchii</i>		Kitahara et al. 1957a,b
51	<i>Lactobacillus</i>	<i>iners</i>		Falsen et al. 1999
52	<i>Lactobacillus</i>	<i>inguviel</i>		Baele et al. 2003
53	<i>Lactobacillus</i>	<i>intestinalis</i>		(ex Hemme 1974) Fujisawa et al. 1990
54	<i>Lactobacillus</i>	<i>jensenii</i>		Gasser et al. 1970
55	<i>Lactobacillus</i>	<i>johnsonii</i>		Fujisawa et al. 1992
	<i>Lactobacillus</i>	"jugurta"		Orla-Jensen, 1919
56	<i>Lactobacillus</i>	<i>kalicensis</i>		Roos et al. 2005
	" <i>Lactobacillus</i> "	<i>kandleri</i>		Holzapfel and van Wyk 1982, 1983
57	<i>Lactobacillus</i>	<i>kefiranciferis</i>		Fujisawa et al. 1988 emend. Vancanneyt et al. 2004
		<i>kefiranciferens</i>	<i>kefiranciferens</i>	Fujisawa et al. 1988
		<i>kefiranciferens</i>	<i>kefirgranum</i>	(Takizawa et al. 1994) Vancanneyt et al. 2004
	<i>Lactobacillus</i>	" <i>kefirgranum</i> "		Takizawa et al. 1994
58	<i>Lactobacillus</i>	<i>kefirii</i>		Kandler and Kunath 1983a,b
59	<i>Lactobacillus</i>	<i>kimchii</i>		Yoon et al. 2000
60	<i>Lactobacillus</i>	<i>kitasatoris</i>		Mukai et al. 2003
61	<i>Lactobacillus</i>	<i>kunkeei</i>		Edwards et al. 1998a,b
	<i>Lactobacillus</i>	" <i>lactis</i> "		(Orla-Jensen 1919) Bergey et al. 1934
	<i>Lactobacillus</i>	" <i>leichtmannii</i> "		(Henneberg 1903) Bergey et al. 1923
62	<i>Lactobacillus</i>	<i>lindneri</i>		(ex Henneberg 1901) Back et al. 1996, 1997
63	<i>Lactobacillus</i>	<i>malefermentans</i>		(ex Russell and Walker 1953) Farrow et al. 1988, 1989
64	<i>Lactobacillus</i>	<i>mai</i>		(Carr and Davies 1970) Kaneuchi et al. 1988
		<i>mai</i>	<i>mai</i>	(Carr and Davies 1970) Kaneuchi et al. 1988
		<i>mai</i>	<i>yamanashiensis</i>	Nonomura 1983, Kaneuchi et al. 1988
	" <i>Lactobacillus</i> "	" <i>mellaromicus</i> "		Müller et al. 1974
65	<i>Lactobacillus</i>	<i>maniholivorans</i>		Morion-Guyot et al. 1998
66	<i>Lactobacillus</i>	<i>mindensis</i>		Ehrmann et al. 2003
	" <i>Lactobacillus</i> "	" <i>minor</i> "		Kandler et al. 1983a,c
	" <i>Lactobacillus</i> "	<i>minutus</i>		(Hauduroy et al. 1937) Moore and Holdeman 1972; Olsen et al. 1991
67	<i>Lactobacillus</i>	<i>mucosae</i>		Roos et al. 2000
68	<i>Lactobacillus</i>	<i>murinus</i>		Hemme et al. 1980, 1982
69	<i>Lactobacillus</i>	<i>negevii</i>		Edwards et al. 2000
70	<i>Lactobacillus</i>	<i>namurensis</i>		Scheinrnick et al. 2007
71	<i>Lactobacillus</i>	<i>nantensis</i>		Valcheva et al. 2006
72	<i>Lactobacillus</i>	<i>oligofermentans</i>		Koort et al. 2005a,b

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
<i>Lactobacillus fructivorans</i> (Weiss et al. 1983a)	acidophilus	A	38-40	Lys-D-Asp	DL
	buchneri	C	38-40	Lys-D-Asp	DL
	buchneri	C	39-41	Lys-D-Asp	DL
	buchneri	B	35-38	Lys-D-Asp	DL
	acidophilus	A	34-35	Lys-D-Asp	L
	reuteri	C	49-50	NA	NA
	acidophilus	B	33-35	Lys-D-Asp	DL
	acidophilus	B	35-37	Lys-D-Asp	D
	acidophilus	A	33-35	Lys-D-Asp	DL
<i>Lactobacillus helveticus</i> (Simonds et al. 1971)	acidophilus	A	NA	NA	NA
	acidophilus	A	35-36	Lys-D-Asp	DL
<i>Weissella kandleri</i> (Collins et al. 1993, 1994)	acidophilus	A	34-38	NA	DL
	acidophilus	A	34-38	NA	DL
	acidophilus	A	34-38	NA	DL
<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefiranofaciens</i> (Van Canneyt et al. 2004)	acidophilus	A	34-38	no mDAP	DL
	buchneri	C	41-42	Lys-D-Asp	DL
	plantarum	B	35	NA	DL
	acidophilus	B	37-40	NA	DL
	buchneri	C	NA	Lys-D-Asp	L
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> (Weiss et al. 1983b, 1984)	acidophilus	A	50	NA	NA
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> (Weiss et al. 1983b, 1984)	acidophilus	A	51	NA	NA
	buchneri	C	35	Lys-D-Asp	DL
	plantarum	C	41-42	Lys-D-Asp	NA
	salivarius	A	32-34	DAP	L
	salivarius	A	32-34	DAP	L
	salivarius	A	32-34	mDAP	L
<i>Carnobacterium piscicola</i> (Mora et al. 2003) <i>Carnobacterium maltaromaticum</i> (Mora et al. 2003)	casei	A	48-49	NA	L
	plantarum	A	37-38	Lys-D-Asp	DL
<i>Lactobacillus viridescens</i> subsp. <i>minor</i> ; <i>Weissella minor</i> (Collins et al. 1993, 1994)	reuteri	C	46-47	Orn-D-Asp	DL
<i>Atopobium minutum</i> (Collins and Wallbanks 1992, 1993)	salivarius	B	43-44	Lys-D-Asp	L
	salivarius	A	NA	NA	DL
	buchneri	C	52	NA	DL
	plantarum	B	38.6	NA	DL
	reuteri	C	35.3-39.9	NA	DL (D 30%)

Table 2.2 continued

Number	Genus	Species	Subspecies	References
73	<i>Lactobacillus</i>	<i>oris</i>		Farrow and Collins 1988
74	<i>Lactobacillus</i>	<i>paris</i>		Wiese et al. 1996
75	<i>Lactobacillus</i>	<i>pantheris</i>		Liu and Dong 2002
76	<i>Lactobacillus</i>	<i>parabrevi</i>		Vancanneyt et al. 2006b
77	<i>Lactobacillus</i>	<i>parabuchneri</i>		Farrow et al. 1988, 1989
		<i>parabuchneri</i>	<i>parabuchneri</i>	Farrow et al. 1988, 1989
		<i>parabuchneri</i>	<i>ferintoshensis</i>	Vancanneyt et al. 2005a
78	<i>Lactobacillus</i>	<i>paracasei</i>	<i>paracasei</i>	Collins et al. 1989b
		<i>paracasei</i>		Collins et al. 1989b
		<i>paracasei</i>	<i>tolerans</i>	(Abo-Elnaga and Kandler 1965b) Collins et al. 1989b
79	<i>Lactobacillus</i>	<i>paracollinoides</i>		Suzuki et al. 2004
80	<i>Lactobacillus</i>	<i>parafamaginis</i>		Endo and Okada 2007b
81	<i>Lactobacillus</i>	<i>parakefirl</i>		Takizawa et al. 1994
82	<i>Lactobacillus</i>	<i>paralimentarius</i>		Cai et al. 1999
83	<i>Lactobacillus</i>	<i>paraplantarum</i>		Curk et al. 1996
	<i>Lactobacillus</i>	" <i>pastorianus</i> "		Van Laer, 1892
84	<i>Lactobacillus</i>	<i>peritus</i>		(ex Fred et al. 1921) Zanoni et al. 1987
85	<i>Lactobacillus</i>	<i>perolens</i>		Back et al. 1999, 2000
	" <i>Lactobacillus</i> "	" <i>piscicola</i> "		Hsu et al. 1984
86	<i>Lactobacillus</i>	<i>plantarum</i>		(Orla-Jensen 1919) Bergey et al. 1923
		<i>plantarum</i>	<i>argentoratensis</i>	Brügel et al. 2005
		<i>plantarum</i>	<i>planterum</i>	(Orla-Jensen 1919) Bergey et al. 1923; Brügel et al. 2005
87	<i>Lactobacillus</i>	<i>pontis</i>		Vogel et al. 1994
88	<i>Lactobacillus</i>	<i>psittaci</i>		Lawson et al. 2001b
89	<i>Lactobacillus</i>	<i>remini</i>		Chenoll et al. 2006a
90	<i>Lactobacillus</i>	<i>reuteri</i>		Kandler et al. 1980, 1982
91	<i>Lactobacillus</i>	<i>rhamnosus</i>		(Hansen 1968) Collins et al. 1989b
	" <i>Lactobacillus</i> "	" <i>rimae</i> "		Olsen et al. 1991
92	<i>Lactobacillus</i>	<i>rogosae</i>		Höldeman and Moore 1974
93	<i>Lactobacillus</i>	<i>rossiae</i>		Corsetti et al. 2005
94	<i>Lactobacillus</i>	<i>ruminis</i>		Sharpe et al. 1973
95	<i>Lactobacillus</i>	<i>saeriment</i>		Pedersen and Roos 2004
96	<i>Lactobacillus</i>	<i>sakai</i>		Katagiri et al. 1934 emend. Klein et al. 1996
		<i>sakai</i>	<i>carnosus</i>	Torriani et al. 1996; Koort et al. 2004
		<i>sakai</i>	<i>sakai</i>	Katagiri et al. 1934 emend. Klein et al. 1996
97	<i>Lactobacillus</i>	<i>salivarus</i>		Rogosa et al. 1953
		<i>salivarus</i>	<i>salicinlus</i>	Rogosa et al. 1953 emend. Li et al. 2006
		<i>salivarus</i>	<i>salivarus</i>	Rogosa et al. 1953 emend. Li et al. 2006
98	<i>Lactobacillus</i>	<i>sanfranciscensis</i>		(ex Kline and Sugihara 1971) Weiss and Schüffler 1984a,b
99	<i>Lactobacillus</i>	<i>satsumensis</i>		Endo and Okada 2005
100	<i>Lactobacillus</i>	<i>secaliphilus</i>		Ehrmann et al. 2007
101	<i>Lactobacillus</i>	<i>sharpae</i>		Weiss et al. 1981, 1982

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G + C content	Peptidoglycan type	Lactic acid type
<i>reuteri</i>	C	49-51	Orn-D-Asp	DL	
<i>reuteri</i>	C	49-51	Lys-D-Asp	DL	
<i>casei</i>	A	52-53	NA	D	
<i>buchneri</i>	C	49	NA	DL	
<i>buchneri</i>	C	44	Lys-D-Asp	NA	
<i>buchneri</i>	C	44	Lys-D-Asp	NA	
<i>buchneri</i>	C	43	NA	DL	
<i>casei</i>	B	45-47	Lys-D-Asp	L	
<i>casei</i>	B	45-47	Lys-D-Asp	L	
<i>casei</i>	B	45-47	Lys-D-Asp	L	
<i>plantarum</i>	C	44-45	NA	D	
<i>buchneri</i>	B	40	no mDAP	DL (D< 70%)	
<i>buchneri</i>	C	41-42	NA	L	
<i>plantarum</i>	B	37-38	NA	NA	
<i>plantarum</i>	B	44-45	DAP	DL	
<i>plantarum</i>	C	NA	NA	NA	
<i>Lactobacillus paracasei</i> (Ehrmann and Vogel 2005)					
<i>plantarum</i>	B	46-47	DAP	DL	
<i>perotens</i>	B	49-53	Lys-D-Asp	L	
<i>Carnobacterium piscicola</i> (Collins et al., 1987), <i>Carnobacterium maltaromaticus</i> (Mora et al. 2003)					
<i>plantarum</i>	B	44-46	DAP	DL	
<i>plantarum</i>	B	44-46	NA	DL	
<i>plantarum</i>	B	44-46	DAP	DL	
<i>reuteri</i>	C	53-56	Orn-D-Asp	DL	
<i>acidophilus</i>	C	NA	NA	NA	
<i>casei</i>	B	NA	L-Lys-D-Asp	DL	
<i>reuteri</i>	C	40-42	Lys-D-Asp	DL	
<i>casei</i>	B	45-47	Lys-D-Asp	L	
<i>Atopobium rimae</i> (Collins and Wallbanks 1992,1993)	NA	NA	NA	NA	NA
Taxonomic status unclear due to lack of type strain. (Félix et al. 2004)					
<i>reuteri</i>	C	44-45	Lys-Ser-Ala	DL	
<i>salivarus</i>	A	44-47	DAP	L	
<i>salivarus</i>	A	42-43	DAP	DL	
<i>casei</i>	B				
<i>casei</i>	B	42-44	NA	DL	
<i>casei</i>	B	42-44	NA	DL	
<i>salivarus</i>	A	34-36	Lys-D-Asp	L	
<i>salivarus</i>	A	NA	NA	NA	
<i>salivarus</i>	A	34-36	Lys-D-Asp	L	
<i>buchneri</i>	C	36-38	Lys-Ala	DL	
<i>salivarus</i>	A	39-41	DAP	L	
<i>reuteri</i>	B	48	L-Lys-D-Asp	L (D 5%)	
<i>casei</i>	A	53	DAP	L	

Table 2.2 *continued*

Number	Genus	Species	Subspecies	References
102	<i>Lactobacillus</i>	<i>slifrinis</i>		Aslam <i>et al.</i> 2006
	<i>Lactobacillus</i>	" <i>sobrinus</i> "		Konstantinov <i>et al.</i> 2008
103	<i>Lactobacillus</i>	<i>spicheri</i>		Meroth <i>et al.</i> 2004a,b
104	<i>Lactobacillus</i>	<i>suebicus</i>		Kleynmans <i>et al.</i> 1989
	<i>Lactobacillus</i>	" <i>suntoryeus</i> "		Cachat and Priest 2005
105	<i>Lactobacillus</i>	<i>thailandensis</i>		Tanasupawat <i>et al.</i> 2007
	<i>Lactobacillus</i>	" <i>thermotolerans</i> "		Niamsup <i>et al.</i> 2003
	<i>Lactobacillus</i>	" <i>tinichodes</i> "		Formacion <i>et al.</i> 1949
	<i>Lactobacillus</i>	" <i>luccei</i> "		Chenoll <i>et al.</i> 2006b
	" <i>Lactobacillus</i> "	<i>ulli</i>		Olsen <i>et al.</i> 1991
106	<i>Lactobacillus</i>	<i>ultunensis</i>		Roos <i>et al.</i> 2005
107	<i>Lactobacillus</i>	<i>vaccinostercis</i>		Okada <i>et al.</i> 1979; Kozaki and Okada 1983 emend. Dellaglio <i>et al.</i> 2005
108	<i>Lactobacillus</i>	<i>vaginensis</i>		Embley <i>et al.</i> 1989
109	<i>Lactobacillus</i>	<i>versmoldensis</i>		Kröckel <i>et al.</i> 2003
110	<i>Lactobacillus</i>	<i>vinif</i>		Rodas <i>et al.</i> 2006
	" <i>Lactobacillus</i> "	<i>viridescens</i>		Niven and Evans 1957
111	<i>Lactobacillus</i>	<i>vitiinus</i>		Sharpe <i>et al.</i> 1973
	" <i>Lactobacillus</i> "	" <i>xylosus</i> "		Kitahara 1938
	<i>Lactobacillus</i>	" <i>yamanashiensis</i> "		Nonomura 1963
112	<i>Lactobacillus</i>	<i>zeae</i>		(ex Kurnetsov 1959) Dicks <i>et al.</i> 1996
113	<i>Lactobacillus</i>	<i>zymae</i>		Vancanneyt <i>et al.</i> 2005b

SType of glucose fermentation as defined by Hammes and Vogel (1995) and Hammes and Hertel (2003): A = homofermentative, B = facultatively heterofermentative, C = obligately heterofermentative, NA = not available.

sons the genus today is still considered divided in the same three major groups, namely group I (obligately homofermentative *lactobacilli*), group II (facultatively homofermentative) and group III (obligately heterofermentative) (Tables 2.1 and 2.2). In addition, the accumulated knowledge on their sugar fermentation patterns created a solid basis on which further research was carried out, including other metabolic properties of the *lactobacilli*, such as proteolytic and lipolytic activities, which are equally important in food applications. These aspects are further discussed below.

### Carbon sources metabolism in *lactobacilli*

#### Lactose fermentation

Lactose fermentation is by far the most studied disaccharide metabolism in lactic acid bacteria, since it is the major carbohydrate of milk. As shown for *Lactobacillus casei*, lactose is taken up via the phosphoenolpyruvate-dependent phosphotransferase system (PTS) and enters the cytoplasm as lactose phosphate (Chassy and Alpert, 1989). Lactose phosphate is cleaved by phospho- $\beta$ -D-galactosidase (P- $\beta$ -gal) to yield glucose and galactose-6-phosphate. Glucose is

Current name (reference)	Phylogenetic group	Metabolism type §	Mol% G+C content	Peptidoglycan type	Lactic acid type
<i>Lactobacillus delbrueckii</i> subsp. <i>reuteri</i> (Kandler et al. 1983)	C	44.5	L-Lys-D-Glu-L-Ala	NA	
<i>Lactobacillus amylovorus</i> (Mijanen et al. 2008)	acidophilus	B	35-36	NA	DL
<i>Lactobacillus buchneri</i> (Kandler et al. 1983)	buchneri	B	55	Lys-D-Asp	DL
<i>Lactobacillus reuteri</i> (Kandler et al. 1983)	reuteri	C	40-41	DAP	DL
<i>Lactobacillus helveticus</i> (Naser et al. 2006a)	acidophilus	A	NA	NA	NA
<i>Lactobacillus casei</i> (Fell et al. 2005)	casei	A	49-50	no mDAP	DL
<i>Lactobacillus reuteri</i> (Fell et al. 2005)	reuteri	C	49-51	no mDAP; Lys-Asp	DL
<i>Lactobacillus fructivorans</i> (Weiss et al. 1983a)	buchneri	C	NA	NA	NA
not yet validated	plantarium	A	ND	L-Lys-Gly-D-Asp	DL
<i>Olsenella uti</i> (Dewhurst et al. 2001)	acidophilus	A	35-36	Lys-D-Asp	DL
	reuteri	C	36-37	DAP	NA
	reuteri	C	38-41	Om-D-Asp	NA
	plantarium	A	40-41	NA	L
<i>Weissella viridescens</i> (Collins et al. 1983, 1984)	salivarius	B	39.4	L-Lys-D-Asp	DL
	vulnificus	A	34-37	mDAP	DL
	catenaformis				
<i>Lactococcus lactis</i> subsp. <i>lactis</i> (Schleifer et al. 1985)					
<i>Lactobacillus rhamnosus</i> (Kaneuchi et al. 1988)	salivarius	A	48-49	Lys-D-Asp	L
	casei	B	53-54	NA	DL
	buchneri	C			

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phosphorylated by glucokinase and metabolized through either the glycolytic pathway or the pentose phosphate pathway. Galactose-6-phosphate is metabolized through the tagatose-6-phosphate pathway (Bisset and Anderson, 1974), while the Leloir pathway is used by galactose-fermenting lactic acid bacteria, which transport galactose with a permease and which lack the galactose-PTS (Konings et al. 1989). The enzyme systems of lactose-PTS and P- $\beta$ -gal are generally inducible, and repressed by glucose (Kandler, 1983). An equally common way for lactic acid bacteria to metabolize lactose is by means of a lactose carrier (permease) and subsequent cleavage by  $\beta$ -galactosidase ( $\beta$ -gal) to yield glucose and galactose, which may again enter the two major pathways (McKay et al., 1970; Bhowmik and Marth,

1990). Some of the thermophilic lactobacilli, such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis* and *Lactobacillus acidophilus*, only metabolize the glucose moiety, after transport of lactose and cleavage by  $\beta$ -gal, while galactose is excreted into the medium (Hickey et al., 1986; Hultåns and Morris, 1987).

#### Glucose fermentation

For glucose fermentation two major pathways occur in lactic acid bacteria. The Embden-Meyerhof-Parnas pathway (glycolysis) is used by all lactic acid bacteria, except leuconostocs, group III lactobacilli (obligately heterofermentative species), oenococci and weissellas. It is characterized by the formation of fructose-1,6-diphosphate (FDP), which is split by the

FDP aldolase into dihydroxyacetone-phosphate (DHAP) and glycerinaldehyde-3-phosphate (GAP). GAP (and DHAP via GAP) is then converted to pyruvate in a metabolic sequence including substrate level phosphorylation. One mole of glucose results in 2 moles of lactic acid and a net gain of 2 ATP. The glycolysis pathway is used by the homofermentative lactic acid bacteria. The other main fermentation pathway is the pentose phosphate pathway. The key step is the phosphoketolase split of xylulose-5-phosphate to glycerinaldehyde-3-phosphate (GAP) and acetyl-phosphate. GAP is then converted to lactate, while acetyl-phosphate to acetate and ethanol. This pathway is used by the heterofermentative lactic acid bacteria. Heterolactic fermentation gives 1 mole each of lactic acid, ethanol and  $\text{CO}_2$  and 1 ATP per mole of glucose. It should be noted that glycolysis may lead to a heterolactic fermentation (meaning significant amounts of other end-products besides lactic acid) under certain conditions, and some lactic acid bacteria, regarded as homofermentative, use the pentose phosphate pathway when metabolizing certain substrates (Axelson, 1998).

Among lactic acid bacteria, those found in sourdough fermentations belong mainly to the heterofermentative *Lactobacilli*, which catabolize glucose via the pentose phosphate pathway. Under micro-aerophilic conditions, both oxygen and fructose can be used as electron acceptors. This gives rise to the formation of additional metabolites such as acetate and mannitol (Hammes and Gänzle, 1998).

#### Maltose fermentation

In sourdough, maltose is the most abundant fermentable carbohydrate, and hence maltose catabolism is a key process during fermentation. Microbial associations of maltose-positive and maltose-negative lactic acid bacteria strains are typical for sourdoughs dominated by *Lactobacillus sanfranciscensis* (Gobbetti, 1998). In *L. sanfranciscensis*, *Lactobacillus reuteri* and *Lactobacillus fermentum* a constitutive intracellular maltose phosphorylase catalyses the phosphorolytic cleavage of maltose, yielding glucose 1-phosphate and glucose (Vogel *et al.*, 1994). Glucose 1-phosphate is then converted by phosphoglucomutase to glu-

ose 6-phosphate, which is further metabolized via the pentose phosphate pathway (Hammes *et al.*, 1996; Vogel *et al.*, 1999). On the other hand, hexokinase activity, which catalyses the conversion of glucose to glucose-6-phosphate, is virtually absent in cells growing exponentially in maltose-containing media, and thus the non-phosphorylated glucose becomes excreted in the medium in a molar ratio with maltose of about 1:1 (Stolz *et al.*, 1993; Gobbetti *et al.*, 1994). It has been shown, however, that no glucose accumulation occurred in the fermentation broth, and no maltose phosphorylase activity could be detected in cell extracts prepared from cells grown in the presence of both maltose and fructose, suggesting that in the presence of both maltose and fructose in the medium, induction of hexokinase activity does occur (De Vuyst *et al.*, 2003). Similarly, in experiments performed with growing cells of *L. sanfranciscensis*, no significant accumulation of glucose was observed in the medium as that reported for resting cells of *L. sanfranciscensis*, *L. reuteri*, and *Lactobacillus pontis* (Neubauer *et al.*, 1994; Stolz *et al.*, 1995a, b). It is also believed that hexokinase activity is induced in the presence of glucose or fructose in the medium (Stolz *et al.*, 1996).

#### Fructose fermentation

*L. sanfranciscensis* and *L. pontis* are able to use fructose as carbon source; however, in the presence of maltose they use it mainly as an electron acceptor and fructose is reduced to mannitol (Stolz *et al.*, 1995a; Hammes *et al.*, 1999; Wolftum and Vogel, 1999). According to Röcken and Voyséy (1995), oxygen was proved to be the preferred hydrogen acceptor for the *L. sanfranciscensis* strains. When oxygen is depleted, fructose is used as an electron acceptor (Gobbetti *et al.*, 1995; Stolz *et al.*, 1995a). Through the reduction of fructose to mannitol, extra ATP is produced via the acetate kinase reaction, and thus maltose-fructose co-metabolism yields shorter lag phase and higher growth rate and biomass production. It has been shown that at a molar ratio of 4:1 (fructose-maltose), acetic acid is the main product (Martinez-Anaya *et al.*, 1994; Gobbetti *et al.*, 1995, 2000; Stolz *et al.*, 1995a). *L. sanfranciscensis* converts stoichiometrically fructose to mannitol,

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while *L. pontis* produces small amounts of lactic acid and ethanol (Hammes et al., 1996).

#### Pentose fermentation

As far as pentose fermentation is concerned (Kandler, 1983; Posthuma et al., 2002), despite some strain and species differences, group II and group III lactobacilli are pentose positive. In general, specific permeases are used to transport the sugars into the cell. The pentoses are then phosphorylated and converted by epimerases or isomerases to ribulose 5-phosphate or xylulose 5-phosphate, respectively, which can be metabolized by the lower half of the pentose phosphate pathway (Kandler, 1983).

#### Citrate fermentation

Citrate, which is present in many raw materials such as milk, vegetables, etc., can also serve as energy source for lactic acid bacteria. It is generally accepted that next to carbohydrates, citrate metabolism plays an important role in food fermentations. The ability of lactic acid bacteria to metabolize citrate is invariably linked to endogenous plasmid that contains the gene encoding the transporter, which is responsible for citrate uptake from the medium (Hugenholz, 1993).

Within the cell, citrate is initially converted by the citrate lyase to acetate and oxaloacetate. Oxaloacetate is then decarboxylated to pyruvate. According to the intracellular enzyme pool, pyruvate may be then converted (i) to acetyl CoA (via the pyruvate dehydrogenase complex), which leads to acetate (via the acetate kinase) and acetaldehyde/ethanol formation (via the alcohol dehydrogenase); (ii) to formate (via the pyruvate formate lyase); (iii) to alpha-acetolactate (via the acetolactate synthase), which leads to acetoin (acetolactate decarboxylase), and diacetyl and 2,3-butanediol (via the diacetyl/acetoin reductase); and finally (iv) to lactate (via the lactate dehydrogenase). The energy is mostly generated from the conversion of acetyl CoA to acetate, meaning that citrate acts as electron acceptor, resulting in a higher production of acetate and ATP probably via the acetate kinase pathway. Additional energy is produced during the initial breakdown of citrate into pyruvate (Hugenholz, 1993). Furthermore, recent studies performed

with *Lactococcus lactis* subsp. *lactis* biovar diacetylactis (Hugenholz, 1993; Hugenholz et al., 1993) and *Leuconostoc oenos* (Marty-Teyssié et al., 1996) indicated that the uptake of citrate is coupled to the generation of a proton-motive force, which was shown to be strong enough to drive the additional ATP synthesis. Some of the products of citrate catabolism, such as diacetyl, acetaldehyde and acetoin, have very distinct aroma properties and influence significantly the quality of fermented foods. For instance, diacetyl determines the aromatic properties of fresh cheese, fermented milk, cream and butter (De Figureoa et al., 1998). The breakdown of citrate results as well in the production of carbon dioxide, which can add to the texture of some fermented dairy products (Kimoto et al., 1999).

It has been shown that several strains of *L. sanfranciscensis* are able to use citrate as electron acceptor in the presence of maltose (Stolz et al., 1995a). On the other hand, co-metabolism of maltose and citrate has not been observed for *L. pontis* (Hammes et al., 1996). According to Gobetti and Corsetti (1996), during co-metabolism of maltose and citrate, lactic acid and acetic acid are initially produced, but when citrate is exhausted, lactic acid and ethanol are the main products. In all cases, maltose serves as carbon source, while citrate as electron acceptor. The production of small amounts of succinate from citrate has been also observed, indicating the presence of citrate lyase, malate dehydrogenase, fumarase and succinate dehydrogenase. A putative citric acid cycle (PCAC) for *L. casei* was recently generated, utilizing the genome sequence and metabolic flux analyses (Diaz-Muniz et al., 2005). Although it was possible to construct a unique PCAC for *L. casei*, its full functionality was unknown. Therefore, the *L. casei* PCAC was evaluated utilizing end product analyses of citric acid catabolism during growth in modified chemically defined media (mCDM), and Cheddar cheese extract (CCE). Results suggest that under energy source excess and limitation in mCDM this micro-organism produces mainly L-lactic acid and acetic acid, respectively. Both organic acids were produced in CCE. Additional end products include D-lactic acid, acetoin, formic acid, ethanol, and diacetyl. Production of

succinic acid, malic acid, and butanediol was not observed. It is thus concluded that under conditions similar to those present in ripening cheese, citric acid is converted to acetic acid, L/D-lactic acid, acetoin, diacetyl, ethanol, and formic acid. The PCAC suggests that conversion of the citric acid-derived pyruvic acid into acetic acid instead of lactic acid, may yield two ATPs per molecule of citric acid.

Lactobacilli usually dominate the lactic acid bacteria microflora in naturally fermented sausages. The growth and metabolism of lactic acid bacteria is affected by the presence of oxygen. Usually the carbohydrates are metabolized via glycolysis. However, under certain conditions, the heterofermentative pathway is activated, resulting in undesirable flavour components, i.e. acetate (Jessen, 1995). In the presence of oxygen, metabolites other than those found in anaerobic conditions may be observed. Despite the formation of hydrogen peroxide, which may be formed during the aerobic metabolism of glucose, the yield of lactic acid, acetic acid, acetoin and ethanol are affected. *Lactobacillus plantarum*, which under anaerobic conditions mainly forms lactic acid from glucose, shows a dramatic increase in the production of acetic acid under aerobic conditions, together with small amounts of acetoin (Kröckel, 1995).

### Proteolytic system of lactobacilli

#### Proteolysis

Proteolysis is considered the most complex of the three primary events during food fermentations, the other two being carbohydrate fermentation and lipolysis. It is a general belief that lactic acid bacteria have limited abilities to synthesize amino acids, which are essential for their growth, and most raw food materials contain insufficient amounts of free amino acids and low molecular mass peptides to sustain growth (Law and Kolstadt, 1983; Thomas and Pritchard, 1987). Although they are considered as weak proteolytic bacteria compared with other groups of microorganisms, it has been shown that lactic acid bacteria possess a complex proteolytic system capable of hydrolysing food proteins to peptides and amino

acids (Kunji *et al.*, 1996; Mierau *et al.*, 1997). Furthermore, it is generally accepted that their proteolytic system contributes to the degradation of food protein and hence to the texture, taste and aroma of fermented products (McSweeney and Sørensen, 2000).

The most extensively studied proteolytic system is that of *Lactococcus lactis*, and it serves as a model for all lactic acid bacteria. The second best unravelled proteolytic systems are those of *Lactobacillus* species, most notably *Lactobacillus helveticus*, *Lactobacillus bulgaricus* and *L. casei*. An extracellular membrane-anchored serine proteinase (PrtP) is an essential component of this system. PrtP exists in at least two variants with somewhat different specificities in the degradation of milk casein. The gene encoding PrtP has been cloned and sequenced for a number of *Lactobacillus paracasei* (Holck and Nes, 1992) and *L. bulgaricus* (Gilbert *et al.*, 1996) strains. The *L. paracasei* enzyme shows more than 95% similarity to the lactococcal one, while the *L. delbrueckii* proteinase shows up to 40% identity over the first 820 residues when compared to the lactococcal enzymes; however the C-terminal part does not share any homology. Studies have indicated that *L. helveticus* may contain two proteinases with different substrate specificities (Gilbert *et al.*, 1997), while a cell envelope-associated proteinase gene (*prtH*) was identified in *L. helveticus* CNRZ32, with a deduced amino acid sequence having significant identity (45%) to that of the lactococcal PrtP proteinases (Pederson *et al.*, 1999).

The majority of sourdough lactic acid bacteria does not exhibit cell wall-associated proteinase activity (Pepe *et al.*, 2003; Vermeulen *et al.*, 2005). Generally, a comparable extent of protein degradation is observed in wheat sourdough and in chemically acidified dough (Thiele *et al.*, 2004; Loponen *et al.*, 2004). However, several strains of sourdough lactic acid bacteria strains exhibiting proteolytic activity were characterized (Gobbetti *et al.*, 1996a; Di Cagno *et al.*, 2002; Pepe *et al.*, 2003) and a contribution of selected lactic acid bacteria to proteolysis could be demonstrated by analysis of the degradation of albumins, globulins, and gliadins in wheat sourdoughs (Di Cagno *et al.*, 2002; Pepe *et al.*,

2003; Zotta *et al.*, 2006). The analysis of peptide and amino acid levels in wheat sourdoughs indicate that *L. sanfranciscensis* preferably utilizes peptides during growth in sourdough (Thiele *et al.*, 2004). Comparable to *Lactococcus lactis* and *L. plantarum*, *L. sanfranciscensis* expresses transport systems for oligo- and dipeptides (Vermeulen *et al.*, 2005) and peptides are hydrolysed by intracellular peptidases, several of which have been characterized at the biochemical or genetic level (Gobboff *et al.*, 1996a; Gallo *et al.*, 2005; Vermeulen *et al.*, 2005). Analysis of the regulation of peptide uptake systems and peptidases during growth of *L. sanfranciscensis* has shown that genes coding for the peptide uptake systems for dipeptides (DtpT) (Foucaud *et al.*, 1995) and an oligopeptide transport system (Opp) were expressed during exponential growth in sourdough and their expression was reduced in stationary phase cells or when the peptide supply in dough was increased (Vermeulen *et al.*, 2005).

#### Amino acid transport

In lactococci, the products of the initial casein degradation (amino acids and peptides) are transported into the cell by transport systems specific for amino acids (Konings *et al.*, 1989), two di- and tripeptides (DtpT and DtpP) (Foucaud *et al.*, 1995) and an oligopeptide transport system (Opp) accepting four to eight residue peptides (Tykkynen *et al.*, 1993). However, little information is available on the transport of casein breakdown products in lactobacilli. Results suggest that the amino acid transport systems in *L. helveticus* are similar to the lactococcal (Nakajima *et al.*, 1998). The gene coding for a branched chain amino acid carrier (bmc) of *L. delbrueckii* subsp. *lactis* has been cloned and sequenced (Stucky *et al.*, 1995), and it is driven by the proton motive force. For the same organism the genes coding for an aromatic acid and a dipeptide transporter, *aroP* and *dppE*, respectively, have also been cloned and sequenced (Kunji *et al.*, 1996). For *L. helveticus*, a homologue of DtpT is specified by a sequence located downstream of *pepN* (Christensen *et al.*, 1995), and transport experiments have shown that substrates, typical for the lactococcal DtpT, are indeed transported

by this organism. Experiments also indicate that an oligopeptide transport system is present in *L. helveticus* (Nakajima *et al.*, 1998). Inside the cell, several peptidases with a wide range of specificity complete the degradation (Christensen *et al.*, 1999).

#### Amino acid metabolism

In addition to proteolysis, bacterial amino acid metabolism contributes to flavour formation during fermentation. In recent years, it has become clear that a number of enzymes are involved in the conversion of amino acids to flavour components. Indeed, the genome sequence analysis of several species of lactic acid bacteria provided insight into the metabolic pathways for amino acid conversion (van Kranenburg *et al.*, 2002). These enzymes may catalyse reactions such as transamination, decarboxylation, and cleavage of the amino acid side chain. Branched-chain amino acids can be transaminated to ketoacids, which then undergo either spontaneous degradation or they are enzymatically converted to the corresponding aldehydes or carboxylic acids (Smit *et al.*, 2000). Amino acid transamination is a key step in the amino acid conversion to aroma compounds by lactic acid bacteria. Indeed, in lactic acid bacteria catabolism of AAAs, BCAAs, and Met is essentially initiated by a transamination reaction since the degradation occurs only in presence of an alpha-ketoacid which is used as amino group acceptor. This was demonstrated in mesophilic lactobacilli such as *L. paracasei*, *L. casei*, *L. plantarum*, *Lactobacillus rhamnosus* (Gummalla and Broadbent, 1996; Tammam *et al.*, 2000) and also in thermophilic lactobacilli such as *L. helveticus*, *L. delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus* (Gummalla and Broadbent, 1999).  $\alpha$ -Ketoglutarate serves as amino acceptor in the transamination reaction of leucine, phenylalanine and other amino acids, and the addition of  $\alpha$ -ketoglutarate strongly increases amino acid conversion of *Lactobacillus sakei* and *L. plantarum* (Yvon *et al.*, 1998; Lartouture *et al.*, 2000). Lactic acid bacteria exhibit glutamate dehydrogenase activity in a strain specific manner. The enzyme catalyses the NAD(P)H-dependent recycling of glutamate to  $\alpha$ -ketoglutarate, and consequently increases the flux through the

transaminase reaction (Gänzle *et al.*, 2007). The catabolism of leucine and phenylalanine was analysed in detail with strains of *L. sakei* and *L. plantarum* (Groot and de Bont, 1998; Lartouture *et al.*, 2000), valine and isoleucine are degraded by comparable metabolic pathways. Cystathione lyase (Cxl) is a key enzyme in the metabolism of methionine and cysteine in lactic acid bacteria. Cystathione- $\gamma$ -lyase was purified and characterized from *L. fermentum* and *L. reuteri* (De Angelis *et al.*, 2002). Vermeulen *et al.* (2003) reported that *L. fermentum*, *L. reuteri*, *L. ponis*, *Lactobacillus parus* and *Lactobacillus mucilensis* but not *L. sanfranciscensis*, *L. plantarum* and *Lactobacillus brevis* expressed genes coding for Cxl. Cxl activity of lactic acid bacteria contributes to the flavour development during cheese ripening (Weimer *et al.*, 1999), and a cysteine uptake system was shown to be essential for oxygen tolerance in *L. fermentum* (Turner *et al.*, 1999) but a possible functional role of cysteine and methionine metabolism in sourdough remains to be determined.

#### Lipolytic system of lactobacilli

Lipolysis is among the principal events occurring during cheese ripening. Free fatty acids can be further converted to methyl ketones, lactones, thioesters, keto and hydroxy acids, which contribute in addition to the free fatty acids to the flavour of the ripened product, while the volatile short chain fatty acids are responsible for the rancid flavour of milk (El Soda *et al.*, 1995). The main lipolytic agents in cheese include the indigenous milk lipoprotein lipase, but also the lipases and esterases produced by the starter and non-starter bacteria, and depending on the cheese variety enzyme preparations added during manufacturing.

To hydrolyse milk fat in milk and cheese, lactic acid bacteria possess esterolytic and lipolytic enzymes capable of hydrolysing a range of esters of FFA, tri-, di-, and monoacylglyceride substrates (Fox and Wallace, 1997). Despite the presence of these enzymes, lactic acid bacteria, especially *Lactococcus* and *Lactobacillus* spp. are generally considered to be weakly lipolytic in comparison to species such as *Pseudomonas*, *Acinetobacter* and *Flavobacterium* (Fox *et al.*, 1993). However,

because of their presence in cheese at high numbers over an extended ripening period, lactic acid bacteria are considered likely to be responsible for the liberation of significant levels of FFA. To date, lipases/esterases of lactic acid bacteria appear to be exclusively intracellular and a number have been identified and characterized (Chich *et al.*, 1997; Castillo *et al.*, 1999; Liu *et al.*, 2001). El-Soda *et al.* (1986) found intracellular esterolytic activities against substrates up to C5:0 in *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis* and *L. acidophilus*, with *L. delbrueckii* subsp. *lactis* and *L. acidophilus* displaying the highest activities. Khalid and Marth (1990) reported the quantitative estimation of the lipolytic activity of *L. casei*, *L. plantarum* and *L. helveticus* towards milk fat, olive oil and tributanoic acid emulsions. The three emulsions were hydrolysed by the lactobacilli with the exception of one *L. casei* strain, which failed to hydrolyse olive oil. According to Lee and Lee (1990), esterolytic and lipolytic enzymes were produced by cell lysis of *L. casei* subsp. *casei* LLG, while *L. fermentum* contains a cell surface-associated esterase specific for C4:0, which can hydrolyse  $\beta$ -naphthyl esters of fatty acids from C2:0 to C10:0 (Gobbetti *et al.*, 1997). Gobbetti *et al.* (1996b) reported the purification of an intracellular lipase from a *L. plantarum* strain isolated from Cheddar cheese, with a molecular mass of 65 kDa, and pH and temperature optima of 7.5 and 35°C, respectively. The enzyme was relatively heat stable to a temperature of 65°C but was irreversibly inactivated on heating to 75°C for 2 min. Hydrolysis of triacylglycerides indicated that the enzyme had highest activity on tributanoic acid, less activity on tridodecanoic and trihexadecanoic acids and no activity on tri-cis-9-octadecenoic acid.

Lipids are only a minor component of wheat and rye flours but have a significant effect on bread quality. *L. sanfranciscensis* is auxotroph for unsaturated fatty acids (Sugihara and Kline, 1975). Unsaturated fatty acids are subject to autoxidation during flour storage, and are oxidized by cereal lipoxygenase activity during dough mixing (Laignel and Dumas, 1984). (E)-2-Nonenal and other aldehydes resulting from lipid oxidation are key aroma compounds in wheat

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and rye bread that impart a 'fatty', 'metallic' or 'green' flavour (Hansen and Schieberle, 2005) and the concentrations of these flavour compounds are significantly reduced during sourdough fermentation (Czerny and Schieberle, 2002). The SC-ADH activity of lactobacilli contributes to the reduction of these flavour compounds during sourdough fermentation, yeasts additionally may exhibit ADH activity in dough.

### The taxonomy of the genus *Lactobacillus*

#### Some taxonomic background

The genus *Lactobacillus* belongs to the large group of lactic acid bacteria which are all Gram-positive non-sporing cocci, coccobacilli or rods, having a DNA base composition of less than 50 mol% G + C. As mentioned before they lack catalase and 'need' a fermentable carbohydrate for growth. The lactic acid bacteria in the broad sense comprise genera such as *Acrococcus*, *Alliococcus*, *Atopobacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Paralactobacillus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. The genus *Bifidobacterium*, *Gardnerella*, *Scardovia* and *Parascardovia* are often also included in this collection, although phylogenetically they belong to the Actinobacteria subdivision (PHYLUM 3), of the Gram-positive Eubacteria (the Firmicutes), comprising also *Propionibacterium*, *Brevibacterium* and the microbacteria. The latter taxa are only very distantly related to the genuine lactic acid bacteria.

The genus *Lactobacillus* belongs phylogenetically to the phylum Firmicutes (Garrity *et al.*, 2004). The family *Lactobacillaceae* comprises the main family in the order *Lactobacillales* which itself belongs to the class *Bacilli*. From the other members of the family mentioned above, the genera *Paralactobacillus* and *Pediococcus* are most noteworthy since species of these genera tend to intermingle phylogenetically with the variety of species of the genus *Lactobacillus*.

This classification was mainly build on the results of 16S rRNA sequence analysis (Taxonomic Outline of the Prokaryotes; Garrity *et al.*, 2004), and as mentioned before does not necessarily

reflect the metabolic diversity discussed above. Equally essential as the 16S rRNA sequencing technique to this scheme, is the species concept (*The ad hoc committee for the re-evaluation of the species definition, in bacteriology*; Stackebrandt *et al.*, 2002). Although the species concept remains subject of animated debates among taxonomists (Rosselló-Móra and Amann, 2001; Rosselló-Móra, 2003; Gevers *et al.*, 2005), it remains the formal unit of bacterial classification and is defined as a monophyletic and genetically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many 'independent' characteristics, and is diagnosable by a discriminative phenotypic property (Rosselló-Móra and Amann, 2001). This phylogenetic concept requires that besides genetic evidence, some phenotypic characteristics will discriminate a possible new species from its closest phylogenetic neighbours. When phenotypic variation in a species is considerable, a species may be further subdivided into subspecies, based on this phenotypic variation; subspecies can but need not be supported by genetic determinants (Rosselló-Móra and Amann, 2001).

In practice, a species is defined by two main genotypic criteria: strains with a total DNA similarity of 70% or higher (relative binding in a hybrid DNA reassociation experiment) and a difference in the melting temperature ( $\Delta T_m$ ) equal to or lower than 5°C, will be considered to belong to a single species; in addition the 16S rRNA gene sequence similarity should not differ more than 3%. As mentioned above, phenotypic features should be sought that confirm the proposed groupings.

DNA-DNA similarity measures are thus still considered the gold standard technique for the delineation of bacterial species. Since this technique is laborious, it is very unpractical for the 113 species of the genus *Lactobacillus*. Therefore, the closest phylogenetic species are often identified by a (partial) 16S rRNA sequencing, which after comparison with the large collection of 16S rRNA sequences available in public databases, allows the identification of the most closely related species for which DNA-DNA hybridizations will need to be set up.

For several reasons, 16S rRNA gene sequencing can never be used as the sole method for species delineation (Stackebrandt and Goebel, 1994).

- 1 A first limit might be that often a single representative strain per species is used for the 16S rRNA analysis, lacking the possibility to position a new isolate in the biological diversity of the species considered.
- 2 The use of partial sequences is also making the result of 16S rRNA sequences less reliable.
- 3 The many sequencing errors present in the reference sequences (often from the early days of sequencing), will also influence the final tree.
- 4 Sequence alignment, essential for sequence similarity calculation, is a highly subjective business. Not only does it rely on a wealth of algorithms, but often manual editing is necessary to 'improve' the result obtained. Critical are the ease one allows the software to create gaps, and the 'cost' defined to extend these gaps.
- 5 Also, in the pairwise calculation of the sequence similarities, gaps can be included or not and phylogenetic corrections can be applied or not.
- 6 The cluster algorithm chosen, as well as the selection of reference sequences included, will also affect the shape of the final tree.
- 7 Finally, as a conserved taxonomic marker, 16S rRNA is not really suitable to study small differences between closely related species.

For these reasons, 16S rRNA sequencing will be useful to frame a new isolate in a well-known phylogenetic scheme, but may not solve the real identification or classification problem. A polyphasic approach (Vandamme *et al.*, 1996a), taking into consideration a variety of information sources, should result in a more reliable identification or classification.

In view of the wide use of lactic acid bacteria in food applications, identification and classification, however, are very important. The discussion whether evolutionary deductions should automatically reflect on nomenclatural

designation (Dellaglio *et al.*, 2004b) is a very relevant one. Nomenclature is essential for proper food labelling and will allow producers to communicate in a formal way about the bacteria they add to foods. Safety aspects, for example, have also been linked to species definition (the QPS principle; [http://www.efsa.europa.eu/EFSA/efsa\\_locale-1178620753812\\_1178620759439.htm](http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620759439.htm)). One could therefore support the automatic link between evolution and speciation (de Quieroz and Gauthier, 1992; Woese, 1998; Cantino 1999), but its automatic translation into nomenclatural designations can be questioned for practical and other reasons.

As an example we could use the *Lactobacillus casei* case. The taxonomic controversy has been going on for quite some time. *Lactobacillus casei* was described by Orla-Jensen in 1919. Hansen and Lessel (1971) choose strain ATCC 393 as the neotype strain based on a limited number of phenotypic traits. DNA-DNA hybridization experiments (Dellaglio *et al.*, 1975) showed that strain ATCC 393<sup>T</sup> had high DNA similarity with the former type strain of 'Lactobacterium zae' (Kuznetsov, 1959) and as such was shown not the best neotype strain for *L. casei*. Using DNA-DNA hybridization experiments Collins *et al.* (1989b) confirmed the separate position of strain ATCC 393<sup>T</sup> and transferred all other *L. casei* strains to a new species *L. paracasei*.

In a first request for opinion Dellaglio *et al.* (1991) proposed strain *Lactobacillus casei* ATCC 334 (not investigated by Collins *et al.*, 1989b), as an alternative neotype strain of *L. casei* in place of ATCC 393<sup>T</sup> and requested the rejection of the name *L. paracasei*. This request was denied by the Judicial Commission of the International Committee on Systematic Bacteriology (Wayne, 1994), as this would create a precedent in replacing a type strain that was officially described and which was still readily available (Wayne, 1994). In 1996, Dicks *et al.* reclassified *L. casei* subsp. 'casei' ATCC 393<sup>T</sup> and 'Lactobacterium zae' ATCC 15820 as *L. zae* nom. rev., and designated strain ATCC 334 as the neotype of *L. casei* subsp. 'casei'. They formerly rejected the name *L. paracasei*. Since this species, however, was validly published, the situation was unclear, as both proposals were standing and a new opinion of the Judicial Commission was required. The